Microencapsulation techniques using ethyl acetate as a dispersed solvent: effects of its extraction rate on the characteristics of PLGA microspheres

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Abstract

Ethyl acetate solvent evaporation and extraction processes were developed to prepare poly(d,l-lactide-co-glycolide) microspheres. The microencapsulation processes first emulsified a polymer-containing ethyl acetate solution with a 1% aqueous polyvinyl alcohol solution (W) to make an oil-in-water (O/W) emulsion. The O:W phase ratio was carefully chosen so as to saturate the W by a small proportion of the dispersed solvent and to form successfully embryonic microspheres without generating polymer precipitates. The effects of the O:W phase ratio on the morphology and size of microspheres were interpreted in terms of the solvent miscibility with water, as well as the influence of the W volume on breakup of the dispersed phase. The extraction rate of ethyl acetate from nascent microspheres was then adjusted by making use of both its miscibility with water and its volatility at atmospheric pressure. Variation of these parameters made it possible to fabricate hollow- or matrix-type microspheres with different size distributions. It was also found that the tendency of microspheres to aggregate on drying was related to the extent of microsphere hydration and the residual ethyl acetate in wet microspheres.

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Keywords: Ethyl acetate; Poly(d,l-lactide-co-glycolide); Microspheres; Solvent extraction rate

1. Introduction

Recent investigations have invested considerable efforts in the microencapsulation of peptides, proteins and antigens into poly(d,l-lactide-co-glycolide) (PLGA) microspheres for their controlled release. The most commonly used microencapsulation technique is based on the concept of solvent evaporation/extraction and employs methylene chloride and water as dispersed and continuous phases, respectively [1].

A variety of methods that rely on the two phases are very well documented in a number of publications. The use of halogenated alkanes, such as methylene chloride and chloroform, however, is not desirable from the viewpoints of environmental and human safety, so they are not recommended for routine manufacturing process. Furthermore, the use of methylene chloride may also impose a problem in obtaining product approval by regulatory agencies. As evidenced by Lupron Depot®, a small amount of methylene chloride remaining in a microsphere product is acceptable by the FDA, but only if the product’s therapeutic benefits clearly outweigh a
Table 1
Examples of non-chlorinated solvents used for preparing PLGA particles

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Preparation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>A salting-out procedure utilizing an electrolyte-saturated continuous phase [2]</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Rapid freezing of a polymeric phase in a liquefied gas, followed by solvent extraction [3]</td>
</tr>
<tr>
<td>Ethyl formate</td>
<td>Water-in-oil-in-water emulsion techniques [4,5]</td>
</tr>
<tr>
<td>N-methyl-2-pyrrolidone dimethyl sulfoxide</td>
<td>In situ solidification of polymeric materials due to dissipation of solvents to external aqueous media [7,8]</td>
</tr>
<tr>
<td>Methylethyl ketone</td>
<td>A solvent extraction technique making use of partial water-miscibility of the solvent [9]</td>
</tr>
<tr>
<td>Phthalic acid diethyl ether</td>
<td>Freeze-drying of the polymeric solution followed by ball milling to produce randomly shaped particles [10]</td>
</tr>
</tbody>
</table>

safety concern over the residual solvent. However, facing a microsphere product to be marketed as preventive medicaments such as vaccine, the regulatory agency may raise a concern over the possible risk that the residual solvent triggers: methylene chloride is a suspected carcinogen and mutagen.

In recognition of this issue, a number of investigations have sought safer solvents (Table 1). Among them, ethyl acetate is considered one of the most preferable solvents. Considering the issues of both environmental/human safety and product approval, ethyl acetate is regarded as a better solvent than dichloromethane. The major physical properties of ethyl acetate and dichloromethane are compared in Table 2. Investigations relating to the effects of ethyl acetate on microsphere quality have not been fully reported in current scientific or patent publications. Therefore, this study focused on the development of a microencapsulation process utilizing ethyl acetate as a dispersed solvent. It also investigated key process parameters that affected the characteristics of PLGA microspheres, such as their morphology, size distribution, and aggregation on drying. Finally, assessment was made of the differences observed with the microspheres prepared from ethyl acetate and methylene chloride.

2. Materials and methods

2.1. Materials

Birmingham Polymers Inc. (Birmingham, AL) was the supplier of poly(d,l-lactide-co-glycolide) with a lactide/glycolide ratio 85:15 (inherent viscosity = 0.28 dl/g in chloroform at 30°C). This polymer was noted as PLGA85:15 in the text. A 88% hydrolyzed polyvinyl alcohol (M_w = 25,000) was obtained from Polysciences Inc. (Warrington, PA). HPLC grade acetone, ethyl acetate, methylene chloride, and N,N-dimethyl formamide were from Fisher Scientific (Malvern, PA).

2.2. Preparation of microspheres

2.2.1. Solvent extraction process with use of different O:W phase ratios

PLGA85:15 (700 mg) was dissolved in 8 ml of

Table 2
Some physical properties of dichloromethane and ethyl acetate

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Bp (°C)</th>
<th>Density at 20°C</th>
<th>Solubility (wt%) at 20–25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Solvent in water</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>39.8</td>
<td>1.3255</td>
<td>1.32</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>76.7</td>
<td>0.9018</td>
<td>8.70</td>
</tr>
</tbody>
</table>

ethyl acetate. A 1% aqueous polyvinyl alcohol solution was selected as a continuous phase, and its volume was 20, 50, 80, or 110 ml. The dispersed phase was then poured into the continuous phase. During the addition, the aqueous phase (W) was stirred at 400 rpm with a magnetic stirrer (400 HPS/VWR Scientific Co.) to produce an oil-in-water (O/W) emulsion. After 2 min, the emulsion was transferred quickly to additional distilled water (W2); the combined total volume of W1 and W2 was always maintained at 200 ml. After 60 min, microspheres were collected by filtration and redispersed in 100 ml of a 0.1% aqueous polyvinyl alcohol solution (W3). The microsphere suspension was stirred for 2 h and was wet sieved (mesh size 355 μm) to remove big microspheres and polymer precipitates. The remaining microspheres were then collected by filtration and dried overnight under vacuum at room temperature.

2.2.2. Solvent evaporation process

Without being transferred to the W2 and W3, a primary O/W1 emulsion was stirred overnight at room temperature. Microspheres were then collected by filtration and vacuum dried. During the microencapsulation process, the O:W1 phase ratio was fixed at 8:20.

2.2.3. Solvent change from ethyl acetate to methylene chloride

PLGA85:15 (700 mg) was dissolved in 8 ml of methylene chloride. To be consistent with the ethyl acetate extraction process described earlier, the polymeric dispersed phase was poured into 20 ml of a 1% aqueous polyvinyl alcohol solution. After 2 min, the emulsion was transferred to 180 ml of distilled water. The O/W emulsion was stirred at 400 rpm for 300 min in order to let methylene chloride partition from polymeric microdroplets and evaporate through the air/liquid interface. Microspheres were then collected by filtration and dried overnight under vacuum at room temperature.

2.3. Observation of various O/W1 and O/(W1+W2) emulsions

The morphology of O/W1 or O/(W1+W2) emulsions with different formulations was monitored during various microencapsulation processes. Aliquots (5 ml) of the emulsions were collected by centrifugation after they were stirred for 15, 40, 150, and 300 min. The samples were then observed under a Zeiss light microscope.

2.4. Measurement of microsphere hydration

At the end of each microencapsulation process, microspheres were collected by filtration, weighed immediately (M1) and after drying to a constant weight (M2). The percentage of microsphere hydration was then calculated by:

\[ \text{Microsphere hydration} \% = \left( \frac{M_1}{M_2} \right) \times 100 \] (1)

2.5. Determination of the residual ethyl acetate in microspheres

Accurately weighed microsphere samples (20.3–44.6 mg) were completely dissolved in 4 ml of N,N-dimethyl formamide, and the solutions were then spiked with an internal standard (acetone). Samples were prepared in triplicate for each microsphere formulation. A Hewlett Packard 5890 gas chromatograph with a flame ionization detector was used in this experiment. The initial oven temperature was set at 35°C for 2 min and then increased to a final temperature of 180°C at the rate of 10°C/min. The injector and detector temperature was maintained at 180 and 260°C, respectively. The HP-1 cross-linked methyl silicone gum capillary column was used as a stationary phase with helium as a carrier gas. Calculation of the concentration of ethyl acetate in microsphere samples was based on the standard calibration curve constructed with 0.0001 to 0.0005 ml of ethyl acetate/ml of N,N-dimethyl formamide.

2.6. Determination of the size distribution of microspheres

The size distribution of microspheres was measured using a Microtrac SRA 150 particle size analyzer (Leeds and Northrup Co., FL). To do so, aliquots (200 mg) of final dried microspheres were dispersed in Isopar G solution (70 ml) by a gentle stirring. In one case, the suspension was loaded...
directly into the particle size analyzer. In the other case, the Isopar G suspension was homogenized for 30 s to deagglomerate microspheres prior to sample loading into the analyzer. The patterns of microsphere size distribution determined by the two methods were compared to study the effect of process variables on microsphere aggregation.

2.7. Scanning electron microscopy (SEM)

The morphology of microspheres was investigated using an Amray 1400 scanning electron microscope (Amray Inc., MA). Their internal structures were revealed as follows: microspheres were mixed with Cole-Parmer® epoxy kit comprising epoxy resin and hardener, allowed to set for 2 h at room temperature, and sliced with a blade (cat. #71960/Electron Microscopy Sciences). Samples were then mounted on aluminum holders and sputter-coated in an argon atmosphere.

3. Results and discussion

When 8 ml of PLGA85:15-containing ethyl acetate solution was poured into 20 or 50 ml of the W₁ phase, the polymeric solution was well disintegrated into microdroplets. The subsequent dilution of the contents to 200 ml with additional distilled water (W₂) converted the microdroplets into solid microspheres. In contrast, when the W₁ volume was 80 ml, microspheres became hardened instantly before transfer to the W₂. At the same time, some of PLGA85:15 became irregular precipitates, rather than microspheres. This caused a reduction in microsphere yield. The further increase of the W₁ to 110 ml provided worse results: during emulsification, about 30% of PLGA85:15 was transformed into precipitates. Moreover, the microspheres tended to aggregate during the filtration of the O/(W₁+W₂) suspension, so they could not be well transferred to the W₂ phase.

After various O/(W₁+W₂) emulsions were stirred for 15 min, the morphology of embryonic microspheres was observed under a light microscope (LM). As illustrated in Fig. 1, the use of the O:W₁ phase ratio of 8:20 resulted in the fabrication of relatively small microspheres. Interestingly, the increase in the W₁ to 50 and 80 ml, without the change in the volume of ethyl acetate (8 ml), yielded bigger microspheres. Especially, some microspheres prepared with use of the phase ratio of 8:80 displayed surface defects and irregularity. It was previously reported that during a methylene chloride O/W emulsion process an increase in microparticle size was observed if the volume of an aqueous phase was increased [11]. However, assessment was not offered to which mechanism could account for the results. In our ethyl acetate extraction microencapsulation process, the effects of the O:W₁ phase ratio on the morphology and size of microspheres were interpreted in terms of the miscibility of ethyl acetate with water and the influence of the W₁ phase volume upon breakup of the dispersed phase. Firstly, 1.93 ml of ethyl acetate is miscible with 20 ml of water since the solvent solubility in water is 8.7 wt% (Table 2). Therefore, upon emulsification of the two phases at the ratio of 8:20, the W₁ can be saturated by only 24.1% of the dispersed solvent (Fig. 2). As a result, the majority of ethyl acetate resided in polymeric microdroplets. The subsequent dilution of the O/W₁ emulsion with additional water (W₂) extracted most of the residual solvent, thereby inducing microsphere hardening. In contrast, as the O:W₁ phase ratio decreased, the fraction of ethyl acetate diffusing into the W₁ increased. For example, 96.5% of ethyl acetate could leach to the W₁ when its volume was 80 ml. The fast leaching of most solvent resulted in immediate microsphere solidification at the first O/W₁ emulsification step. In this case, some microspheres tended to be irregularly shaped so that indentation was observed in their surface. This observation was in good agreement with the earlier reports substantiating that a fast removal of solvents from polymeric droplets affected the surface morphology of microspheres [4,12].

Secondly, attention should be paid to the important aspect that the volume of a continuous phase affects the breakup of a dispersed phase. For stirred tanks, Eq. (2) was proposed to correlate droplet size to mixing conditions [13,14]:

$$\frac{dp}{L} = 0.054We^{-3/5} \left[ 1.0 + 4.42Ca \left( \frac{dp}{L} \right)^{1/3} \right]^{3/5}$$  \hspace{1cm} (2)

If Weber number (We) is written in terms of power per unit mass,
Fig. 1. LM photographs of O/(W₁ + W₂) emulsions taken during the ethyl acetate extraction process. The volume of the W₁ phase was (A) 20, (B) 50, or (C) 80 ml. Magnification: 100× and 400×.

\[ d_p \approx 0.054 \left( \frac{\sigma}{\rho_c \varepsilon} \right)^{3/5} = 0.054 \left[ \frac{\sigma}{\rho_c} \left( \frac{V}{P} \right) \right]^{3/5} \]  

where \( d_p \) is droplet size; \( L \), impeller diameter for agitated tank; \( Ca \), Calabrese number; \( \rho_c \), density of a continuous phase; \( \sigma \), interfacial tension; \( \varepsilon \), energy dissipation; \( P \), power; and \( V \), volume. At the phase ratio 8:20, the interfacial tension between the dis-
persed and continuous phases was reduced by a small portion of the dispersed solvent ethyl acetate that leached to the continuous phase (This conclusion was backed up by the following experiment: both the ethyl acetate-free and ethyl acetate-saturated $W_1$ droplets were spread over a glass surface, and the contact angle between each liquid droplet and the surface was compared. The presence of ethyl acetate in the $W_1$ significantly lowered the contact angle). As described in Eq. (3), the decrease in the interfacial tension gives rise to a reduction in microsphere size. However, the increase in the $W_1$ to 80 ml, while maintaining a constant volume of ethyl acetate, diminished such effect. In this case, PLGA85:15 precipitation concurred with the diffusion of ethyl acetate into the $W_1$ that was not saturated by ethyl acetate. Therefore, breakup of the dispersed phase was not facilitated by the reduction in the interfacial tension between the two phases as noted for the case based on the phase ratio of 8:20. Consequently, larger microspheres were formed. Finally, it should be noted that a magnetic stirrer was used to make $O/W_1$ emulsions throughout this experiment. The mixing technique might provide poor initial distribution of the dispersed phase into the continuous phase, thereby contributing to the formation of polymer precipitates. This elaboration was based on the fact that polymer precipitation took place over a short time scale at the $O:W_1$ phase ratio of 8:80 and 8:110.

One of novel methods to remove an organic solvent from embryonic microspheres is to quench an initial microsphere suspension with a large amount of water. Such a procedure, utilizing ethyl acetate as a dispersed solvent, was briefly mentioned in US patent 5 288 496 [15]. Prior to emulsification, an aqueous phase was mixed with 4.9–11.3 wt% of ethyl acetate, and the resultant phase was then emulsified with a polymer-containing ethyl acetate solution. After embryonic microspheres were produced by this technique, a large amount of water was added to quickly extract the ethyl acetate remaining in the microspheres. The organic/aqueous phase ratio disclosed in the patent ranged from 1:155 to 1:413. In our study, after $O/(W_1 + W_2)$ emulsions were stirred for 1 h, microspheres were collected by filtration and redispersed in the $W_3$ to shorten the time required to harvest microspheres. In an additional effort to develop a microencapsulation process that could avoid using a large volume of extraction water and a big reactor to accommodate such a quenching step, an ethyl acetate evaporation process was also exploited in this study. To do so, an $O/W_1$ (8/20) emulsion was stirred at room temperature without being transferred to the $W_2$ and $W_3$. After evaporation was carried out overnight, discrete and free-flowing microspheres were obtained. This satisfactory result suggested that the continuous phase does not have to be doped with additional ethyl acetate, prior to emulsification with the dispersed phase. In addition, a small amount of water is sufficient to fabricate microspheres successfully. The organic/aqueous phase ratio (8:20) used in this ethyl acetate evaporation process was only 1:2.5. The $O:W_1$ phase ratio was manipulated in a way that only a small portion of the dispersed solvent was utilized to saturate the continuous phase in order to form embryonic microspheres successfully. This simple procedure may be easily tailored for the scale-up of a microencapsulation process for many drugs. The evaporation process likely could be shortened by several methods such as blowing a gas into the emulsion, adjusting temperature, or reducing the pressure of the system. Further investigation of these process parameters is warranted because microsphere
quality can be drastically influenced by these variables.

In the present study, LM photographs taken at the end of the solvent evaporation process revealed that microspheres with different quality were fabricated. They were completely transparent and became much smaller than those produced following the solvent extraction process (Fig. 3). Further analysis of microsphere size distribution confirmed that the two different microencapsulation processes greatly affected microsphere size. The average mean volume size \( M_v \) of the microspheres prepared following the solvent extraction process was 93 \( \mu \text{m} \), whereas that prepared following the solvent evaporation process was 44 \( \mu \text{m} \) (Fig. 4). It is believed that the effect of the microencapsulation processes upon microsphere size originates from the different onset of microsphere solidification. Both microencapsulation processes emulsified the organic phase (8 ml) with the \( W_1 \) (20 ml) and produced temporarily stabilized microdroplets. When the O/W emulsion was diluted with the \( W_2 \) during the solvent extraction process, the ensuing PLGA85:15 precipitation led to the immediate hardening of microspheres. Owing to the rapidity of the microsphere hardening, the subsequent in-liquid drying did not affect its particle size. By contrast, the size of polymeric microdroplets dispersed in the \( W_1 \) decreased with stirring time during the solvent evaporation process. This happened due to the slow leaching of ethyl acetate from the polymeric microdroplets and subsequent inward polymer shrinkage. Under this condition, the solvent diffusion from embryonic microspheres to the continuous phase was preceded by solvent evaporation at the air/liquid interface. In the process of the solvent diffusion, a viscous surface layer around polymeric microdroplets could also impede ethyl acetate transfer to the continuous phase, as previously suggested [16,17].

A similar explanation is proposed for the methyl-ene chloride evaporation process based on the phase ratio of 8:20. Despite a 10-fold increase in the volume of the continuous phase, compared to the

![Fig. 3. LM photographs of microspheres fabricated following (A) the ethyl acetate evaporation and (B) the ethyl acetate extraction processes with the O:W ratio of 8:20. Magnification: 400×.](image-url)
ethyl acetate evaporation, the majority (about 75%) of the solvent still resided in initial polymeric microdroplets (the solubility of methylene chloride in water is 1.32 wt%, as shown in Table 2). As a result, embryonic microspheres were more likely viscous liquid microdroplets at the initial microencapsulation stage and coalesced together to form aggregates without mechanical stirring (Fig. 5A). The evaporation of methylene chloride through the air/liquid interface, followed by the solvent diffusion out of the microdroplets, led to microsphere shrinkage (Fig. 5B–D), as well as hardening [18]. Compared to the ethyl acetate evaporation, evaporation of methylene chloride took place more rapidly due to its lower boiling point (39.8°C).

Both the ethyl acetate evaporation and the extraction processes produced the O/W suspensions in which microspheres were well dispersed (Figs. 1 and 3). Surprisingly, the microspheres displayed different behaviors in response to vacuum drying. The microspheres prepared following the solvent extraction process with the phase ratios of 8:80 had a serious drawback in that they were labile to aggregation on drying (Fig. 6A). In contrast, better microspheres from an aggregation point of view were prepared with the phase ratio 8:20 for both the solvent extraction and evaporation processes (Fig. 6B).

To provide more quantitative comparisons, microsphere aggregation on drying was assessed by the patterns of microsphere size distribution determined by two different methods (After drying, microspheres were gently dispersed in Isopar G solution and their size distribution was analyzed before and after homogenization). The microspheres prepared follow-

Fig. 5. LM photographs of O/W emulsions sampled at (A) 15, (B) 40, (C) 150, and (D) 300 min during the methylene chloride evaporation process. Magnification: 100×.
Fig. 6. SEM micrographs of dried microspheres fabricated following (A) the ethyl acetate extraction with the O:W<sub>i</sub> phase ratio of 8:80 and (B) the ethyl acetate evaporation with the phase ratio of 8:20.

...ing the ethyl acetate evaporation process were relatively well dispersed in the Isopar G solution without the aid of an homogenizer (Fig. 7A). The methylene chloride evaporation permitted by far the easier dispersion of final dried microspheres, and the $M_s$ measured before and after homogenization of the microsphere suspension was 110 and 99 $\mu$m, respectively (Fig. 7B).

As a means to elucidate the aggregation of some microspheres on drying, the degree of microsphere hydration before vacuum drying was determined using Eq. (1). The ethyl acetate extraction process with the phase ratio of 8:20 provided microspheres with the hydration of 145.5(±5.5)%. Increasing the $W_i$ volume to 80 ml enhanced microsphere hydration to 173.9 (±3.5)% (Table 3). If microspheres were prepared following the ethyl acetate or methylene chloride evaporation processes, a significant reduction in microsphere hydration was observed and the resultant microspheres were less prone to aggregation on drying.

GC analysis provided the quantitative data on the residual ethyl acetate in microspheres throughout various microencapsulation stages (Fig. 8). During the solvent extraction process, the initial O:W<sub>i</sub> phase ratio had an effect on the level of ethyl acetate...
Table 3
Effects of process variables on the yield and hydration of microspheres

<table>
<thead>
<tr>
<th>Process Variables</th>
<th>Microsphere wt (g)</th>
<th>Microsphere</th>
<th>Wet</th>
<th>Dried</th>
<th>Yield (%)</th>
<th>Hydration (%)</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a O:W (8:20)</td>
<td>0.901</td>
<td>0.618</td>
<td>88.3</td>
<td>145.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b O:W (8:80)</td>
<td>1.029</td>
<td>0.589</td>
<td>84.1</td>
<td>174.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c O:W (8:200)</td>
<td>0.694</td>
<td>0.629</td>
<td>89.9</td>
<td>110.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d O:W (8:100)</td>
<td>0.664</td>
<td>0.602</td>
<td>86.0</td>
<td>110.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a,b Microspheres were prepared following the ethyl acetate extraction process with the initial phase ratio specified.

cd Ethyl acetate or methylene chloride evaporation process was used to prepare microspheres.

persed in the W₃ (the aggregated microspheres, without being transferred to the W₁, were further subjected to vacuum drying and the amount of the residual ethyl acetate was then determined). This

Fig. 7. Size distribution of microspheres prepared following (A) the ethyl acetate evaporation and (B) the methylene chloride evaporation processes. Microsphere size was analyzed before (hatched bar) and after (filled bar) homogenization of Isopar G microsphere suspensions.

Fig. 8. The residual ethyl acetate in microspheres. (A) During the ethyl acetate extraction process, microsphere samples were prepared after collection from (a) O/(W₁+W₃), (b) O/W₁ suspensions, and (c) after drying. (B) During the ethyl acetate evaporation process, samples were prepared (a) before and (b) after drying.
Fig. 9. SEM micrographs featuring the internal morphology of microspheres prepared following either the ethyl acetate extraction with the O:W \_2 ratio of (A) 8:20 and (B) 8:80, or (C) the ethyl acetate evaporation with the phase ratio of 8:20.
suggested that the wet microspheres were so elastic that they could not offer resistance against microsphere fusion. Compared to the solvent extraction process, the lowest concentration of ethyl acetate was obtained with the microspheres prepared following the solvent evaporation process. The levels of the residual ethyl acetate before and drying were 3.83 ± 0.46 and 2.62 ± 0.41 wt%, respectively (Fig. 8B). The issue of solvent selection for conventional evaporation/extraction processes has been addressed in some publications from standpoints of the mechanism of microsphere formation, as well as environmental and human safety [17,19]. It was proposed that rapid polymer precipitation at the nascent polymeric microdroplet surface was of primary importance for the successful microencapsulation. In addition, the rate of polymer precipitation was reported to depend heavily on solvent properties such as interactions with polymers and water-miscibility. Our results reported in this study provide additional information that, if ethyl acetate was used as a dispersed solvent, polymer precipitation was affected by the organic/aqueous phase ratio. This process parameter influenced to a great extent the onset of PLGA85:15 precipitation and microsphere hardening, which in turn affected many microsphere characteristics as discussed so far.

Fig. 9 shows the internal morphology of microspheres prepared under different process conditions. When microspheres were produced following the ethyl acetate extraction process with the initial phase ratio of 8:80, the resultant microspheres tended to possess a hollow core encased by a nonporous shell layer. The quick leaching of ethyl acetate into the W phase seemed to be responsible for inducing an interfacial polymer deposition immediately and leading to the formation of hollow microspheres. This interpretation is supported by earlier reports that the rate of solvent removal from embryonic polymeric microdroplets determines the morphology of microspheres [9,20]. Moreover, as illustrated by the other SEM micrographs, the morphology of microspheres could also be manipulated to possess a monolithic matrix type by adjusting microencapsulation conditions.

Finally, it is of interest to suggest that the degree of microsphere hydration was also affected by solubility properties of solvents and water (Table 2).

A methylene chloride solvent evaporation process usually results in the fabrication of compact, monolithic matrix type microspheres. During the microencapsulation process, water poorly penetrates into the dispersed phase due to its negligible solubility in methylene chloride (0.2 wt%). Therefore, the resultant microspheres are hardly hydrated at the end of the microencapsulation process and are less prone to aggregation on drying. By contrast, the ethyl acetate evaporation and extraction processes reported in this study produce microspheres with a matrix or hollow structure, depending on the organic/aqueous phase ratio and the rate of the solvent removal from embryonic microdroplets. In addition, since water solubility in ethyl acetate is 3.3 wt%, the polymeric microdroplets can absorb a considerable amount of water during the microencapsulation processes. Therefore, during a drying process, microspheres may become soften due to the residual ethyl acetate and water migrating into the surface of microspheres. As this event leads to the aggregation of adjacent microspheres, a drying method should be taken into deliberation.

4. Conclusion

The disintegration of PLGA85:15-containing ethyl acetate solution into microdroplets was significantly affected by the O:W phase ratio. Depending on the phase ratio, emulsification resulted in the generation of either discrete microdroplets or polymer precipitation. Therefore, in conjunction with a proper mixing technique, the phase ratio should be taken into consideration in order to form embryonic microspheres successfully. Both of the microencapsulation processes reported in this study worked well and featured two essential strategies: (a) adjustment of the O:W phase ratio so as to saturate the W phase with only a minor proportion of the dispersed solvent; and (b) manipulation of the rate of solvent removal from embryonic microspheres. These process variables were found to be critical with regard to polymer precipitation, the morphology and size of microspheres, microsphere hydration, and the residual ethyl acetate in microspheres, as well as microsphere aggregation on drying.
References